DISCOVERY AND MORPHOMETRY OF CARDIOMYOCYTE MITOCHONDRIA IN SEMITHIN SECTIONS

T. A. Gevondyan and G. G. Avtandilov

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In electron-microscopic investigations of the myocardium both the qualitative state of the mitochondria and many of their quantitative characteristics are studied [1, 4, 5]. These investigations are carried out as a rule on electron micrographs. A method of discovering cardiomyocyte mitochondria and subjecting them to quantitative analysis in semithin sections 1 μ and 0.6-0.7 μ thick is suggested below.

EXPERIMENTAL METHOD

Pieces of an animal's myocardium were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4, stained with 0.5% uranyl acetate, and embedded in a mixture of Epon and Araldite. Semithin sections 1 μ thick were transferred to a drop of 5% acetone, dried on a hot stage at 80°C, and then incubated (60°C) for 48 h. Before staining, the sections were heated for 10 min on a hot stage (80°C), after which they were stained with a heated mixture of 1% aqueous solution of methylene blue and sodium tetraborate for 2 min. The dye was washed off and the sections rinsed with hot tap water for 10 sec, rinsed for a few seconds with hot distilled water, after which the water was shaken off the glass and the specimen dried on a hot stage. The thickness of 1 μ was specially chosen for the semithin sections with the aim of developing and suggesting to a wide range of morphologists a simple technique for the study of mitochondria, and allowing for the fact that the histological rocking microtome can be adapted for cutting semithin sections with a minimal thickness of 1 μ [2, 3].

Preparations of much better quality can be obtained by staining semithin sections 0.6-0.7 μ thick. These sections also were treated by the scheme described above except that they had to be stained for 3 min and washed with hot tapwater for 1 min. During this time structures of the section except mitochondria are cleared (Fig. 1); mitochondria in the sections are stained deep blue, with clear outlines, so that under the light microscope definite mitochondria can be distinguished even where they are crowded together. Sarcomeres of myofibrils, their A and I disks, the Z band, and the H zone also can be clearly detected. The difficulty of staining semithin sections under 1 μ thick is that numerous small folds are formed during staining, thereby hiding the structures. There are fewer folds if the pieces of tissue are embedded in Araldite. Treating the sections with xylol, hydrochloric acid, or hydrogen peroxide, although reducing fold formation to some extent, impairs and sometimes may even abolish selective staining of the mitochondria and makes the outlines of the stained mitochondria and makes the outlines of the stained mitochondria indistinct. The formula described above enables selectively stained sections with a large surface area, of high quality and free from folds, to be regularly obtained.

EXPERIMENTAL RESULTS

It is important that a section be obtained from each region of a fragment of tissue for selective staining of mitochondria. We know that when different methods of fixation of pieces of tissue from different depths are used, the degree of preservation of the structures differs [7]. In serial sections 1 μ thick stained with methylene blue and obtained from the surface of the fragments down to a depth of 500 μ, we found that mitochondria were selectively stained in the sections starting from the surface of the fragment and down to a depth of 300 μ.

Department of Pathological Anatomy, Central Postgraduate Medical Institute, Moscow
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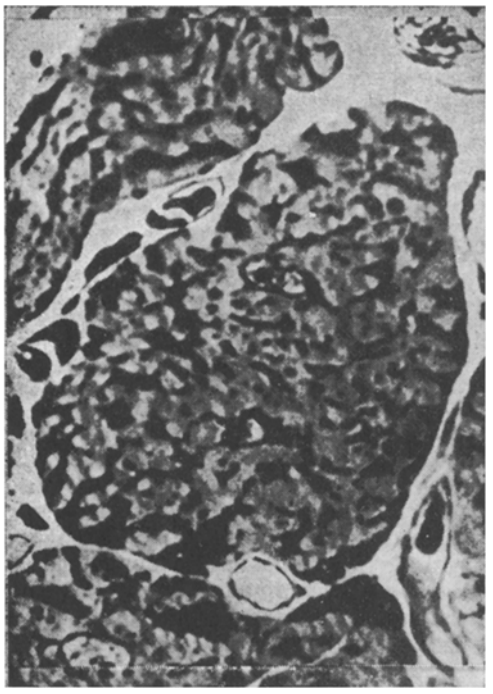

Fig. 1. Transverse section through myocardial fibres of a rat. Stained with methylene blue and sodium tetraboride by method modified by the present authors. 900 ×.

TABLE 1. Changes in Buolk Density of Cardiomyocyte Mitochondria in Semithin Sections (M ± m)

Parameter	Serial No. of section through fiber					
	1	2	3	4	5	6
V_v^m, semithin	0,2724±0,0177	0,3435±0,0087	0,3673±0,0097	0,3802±0,0087	0,3782±0,0114	0,3932±0,0094
V_v^m, ultrathin	0,2239±0,0063	0,2857±0,007	0,3108±0,0078	0,3122±0,0031	0,3139±0,0079	0,3245±0,0028
$\frac{V_v^m, \text{semithin}}{V_v^m, \text{ultrathin}}$	1,2166	1,2023	1,1818	1,2178	1,2048	1,2117

For a comparative analysis of values of stereometric parameters of cardiomyocyte mitochondria, determined in semithin and ultrathin sections, under a magnification of 900 ×, transverse sections through myocardial fibers were photographed in a semithin section under the light microscope, after which these same sections were glued to an Epon-Araldite block, ultrathin sections were cut, and electron micrographs of these same sections of myocardial fibers were prepared with a primary magnification of 3000 ×. Photographs enlarged 20 and 10 times, respectively were prepared from the light-optical and electron-microscopic negatives, and on these enlargements the bulk density of the mitochondrial fraction (V_v^m) was determined by a dot counting method. The period of the morphometric grid was 0.5 cm. The photograph of each cardiomyocyte was measured planimetrically 5 times and mean values calculated. As Table 1 shows, the bulk density of the mitochondria determined in semithin sections was significantly higher in value than the same parameter calculated on electron micrographs, on average by 1.206 ± 0.005 times, whereas the range between the limits did not exceed 3% of the mean value. The reasons why values of V_v^m for semithin sections were higher are the Holmes' effect [6] and the limited resolving power of the light microscope. For semithin sections 0.6-0.7 μ thick, the ratio $V_{v,\text{semithin}}^m / V_{v,\text{ultrathin}}^m$ was 1.199 ± 0.009. Consequently, there was no significant difference compared with the value of the ratio $V_{v,\text{semithin}}^m / V_{v,\text{ultrathin}}^m$ for semithin sections 1 μ thick. Because of the comparatively low resolving power of the light microscope the outlines of separate mitochondria on the photographs were indistinct, small spaces between mitochondira where they were crowded together were invisible, and it is better therefore to use microscopes with television and morphometric attachments.

Consequently, the suggested method of detection and morphometry of cardiomyocyte mitochondria at the light-optical level is simple, rapid, and sufficiently accurate. The method can also be used to carry out the time-consuming preliminary stage of ultrastructural morphometric investigation of mitochondria much more rapidly, thus saving work time on the ultramicrotome and electron microscope.

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MORPHOLOGICAL INVESTIGATION OF GUNSHOT WOUND HEALING BY THE USE OF SEMITHIN SECTIONS

V. V. Grigor'ev, K. K. Zaitseva, and I. D. Kosachev

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The technique of semithin sections (STS) substantially widens opportunities for morphological analysis at the light-optical level. It is used at the present time to study organs and tissues: the nervous system [3], kidneys [4], hematopoietic tissue [8], the eye [2], liver, and gastrointestinal tract [7]. The STS method has not been widely used for the morphological study of wound healing, and the only references to its use for this purpose relate to work by Shekhter et al. [1, 5, 6].

EXPERIMENTAL METHOD

In the present investigation the STS method was used to study the cell composition of granulation tissue during healing of gunshot wounds. Experiments were carried out on 24 rabbits of both sexes weighing 2-2.5 kg. A perforating gunshot wound of the soft tissues of the thigh was inflicted on the animals by the standard method under thiopental anesthesia. The rabbits were killed by air embolism 1, 3, 5, 7, 9, 14, 21, and 28 days after primary surgical treatment (three animals at each time) and material was taken from the region of the wound for morphological investigation. Pieces of tissue were fixed in 2% OsO_4 solution, dehydrated in acetone, and embedded in Epon. STS 1-2 μ thick were obtained on an LKB Ultratome (Sweden). Polychromatic staining of the STS was carried out without removal of the resin in mixture of a 1% solution of methylene blue and a 1% solution of sodium tetraborate, followed by counterstaining with 2% fuchsin solution.*

EXPERIMENTAL RESULTS

The structure of single cells and relations between them were clearly revealed in the granulation tissue on STS. In the initial period of granulation tissue formation (3-7 days) various cells differentiated in it: fibroblasts, macrophages, and neutrophilic granulocytes. The fibroblasts were found in two forms. Cells of the first type, young proliferating fibroblasts, were fusiform, with large oval nuclei containing several nucleoli and with a peri-

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